MECHANISMS OF AMPICILLIN RESISTANCE IN HÆMOPHILUS INFLUENZÆ FROM RESPIRATORY TRACT

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Summary 13 of 100 consecutive isolates of *Hæmophilus influenzæ* obtained from respiratory specimens over the six months to June, 1979, had diminished sensitivity to ampicillin. 6 of the 13 strains produced β-lactamase, whilst the remaining 7 had no evidence of this enzyme, either in whole cells or in extracts prepared by sonication. The minimum inhibitory concentration of ampicillin for the penicillinase-negative strains ranged from 1 mg/l to 8 mg/l on repeated testing with a carefully controlled agar-dilution technique. The findings contrast strongly with those of earlier surveys of the sensitivity of respiratory strains of *H. influenzæ* to ampicillin and confirm the existence of two mechanisms of resistance to ampicillin in the species.

Introduction

THE therapeutic value of ampicillin in the treatment of such respiratory infections as acute exacerbations of bronchitis was supported by the apparently uniform and stable sensitivity of *Hæmophilus influenzæ* to this antibiotic.[1] Before 1974 all strains were believed to be inhibited by 0·5 mg/l or less of ampicillin and therefore tests of sensitivity of respiratory isolates to ampicillin were considered no more than a formality. However, in that year, resistance of *H. influenzæ* to ampicillin, which had been observed in encapsulated strains causing meningitis, was reported in unencapsulated strains isolated from the respiratory tract.[2–4]

The resistance of *H. influenzæ* to ampicillin seemed to be due almost exclusively to the presence of a TEM-type β-lactamase.[5] Thornsberry and colleagues[6] detected this enzyme in 52 of 53 resistant strains collected worldwide. Although the possibility of other mechanisms of resistance was conceded, resistance in *H. influenzæ* other than that mediated by penicillinase production was believed to be rare.[7,8] Hence the suggestion that resistant strains could be identified by tests for the detection of penicillinase alone and that more detailed tests of ampicillin sensitivity were unnecessary.[8,9]

We report here the results of an examination of the nature of ampicillin resistance in 13 strains which were obtained from 100 consecutive isolates of *H. influenzæ* from respiratory specimens.

Material and Methods

Isolates

A sample consisting of 100 consecutive isolates of *H. influenzæ* was collected from specimens of respiratory origin which were received from six different hospitals over the six months to June, 1979. The strains were identified by usual bacteriological techniques including gram-stain characteristics and X-V requirements.[10] Encapsulated strains, recognised by iridescence on Levinthal's agar, were typed by the use of specific antiserum (Wellcome Reagents).

Determination of Sensitivity to Ampicillin

The sensitivity to ampicillin was determined by agar dilution; details of techniques used, including the size of the inoculum and the determination of end point, conformed with those in the recommendations of the International Collaborative Study on Antibiotic Sensitivity Testing.[11] The inocula were prepared from overnight cultures of *H. influenzæ* grown on chocolate agar (Columbia agar [Oxoid] with 8% heated horse blood) and incubated at 35°C in air. Each strain was suspended in 20% nutrient broth in normal saline to obtain a turbidity equivalent to that of Brown's opacity tube no. 4 (Wellcome Reagents). The suspension was diluted to give an inoculum of 10^4 organisms per replicator drop (4 μl), and the size of the inoculum was verified in each case by doing a viable count by the method of Miles and Misra.[12] This suspension was cultured on agar plates containing serial twofold dilutions of ampicillin, and the plates were read after 16 h incubation at 35°C. The sensitivity of each strain was determined by the above procedure on three separate occasions.

Detection of β-lactamase

Whole-cell suspensions and extracts of cells obtained by sonication of each strain with a minimum inhibitory concentration (MIC) of >0·5 mg/l were tested for β-lactamase activity by three methods: the iodometric test of Foley and Perret,[13] the chromogenic cephalosporin 87/312 method,[14] and measurement of ampicillin destruction. In the last test, ampicillin in a final concentration of 0·5 mg/l in phosphate buffer pH 6·5 was incubated overnight with a heavy suspension of whole cells (10^7 organisms/ml) or with sonic extracts. Assay of ampicillin after incubation was done by a plate-diffusion technique using *Sarcina lutea* as the test organism.[15] The sonic extracts were prepared by centrifuging a turbid broth suspension harvested after 4 hours' shaking at 35°C. The centrifuged deposit was washed three times and resuspended in one-tenth of the original volume with sterile distilled water. The concentrated cells were sonicated in an ice bath for six 30 s periods at full output ('Soniprobe' type 7530A, Branson Instruments).

Results

The sensitivity of the 100 isolates to ampicillin is shown in the accompanying table. In 13 strains the MIC of ampicillin was 1 mg/l or more on repeated examinations. 10 of the 13 strains were isolated from specimens taken from outpatients or from patients on admission to hospital, and the remaining 3 were from inpatients in different units in 2 of the hospitals. The presence of a capsule, type b in each case, was shown in 1 of the 13 resistant strains and 3 of the 87 ampicillin-susceptible strains of *H. influenzæ*.

The presence of β-lactamase was detected by all three methods, in both whole cells and sonicated-cell preparations, in 6 of the 13 strains with diminished sensitivity to ampicillin; the encapsulated strain was among these 6 strains. In 4 of the β-lactamase-positive strains the MIC of ampicillin exceeded 8 mg/l, whereas in 2 the

SENSITIVITY TO AMPICILLIN OF 100 ISOLATES OF H. INFLUENZÆ

	Minimum inhibitory concentration (mg/l)						
	>8·0	8·0	4·0	2·0	1·0	0·5	0·25
No. of β-lactamase-positive strains	4	0	2	0	0	0	0
No. of β-lactamase-negative strains	0	1	1	3	2	53	34

MIC was 4 mg/l at the inoculum used. In the remaining 7 strains β-lactamase could not be demonstrated by any of the three methods used to detect destruction of penicillin in either whole-cell suspensions or the concentrated suspension of sonicated cells. The MIC of ampicillin for the 7 β-lactamase-negative strains ranged from 1 mg/l to 8 mg/l.

Discussion

The present study shows a high prevalence in the community of strains of *H. influenzæ* with diminished sensitivity to ampicillin. This prevalence contrasts strongly with the findings of similar surveys carried out in this laboratory and elsewhere before 1974 which showed the absence of strains with any resistance to ampicillin.[16,17] In a later survey, Williams and Cavanagh[4] reported a geographical variation in the prevalence of resistant strains in the midlands of England. The highest prevalence was in Stafford, where 5% of strains were resistant, presumably because of the production of β-lactamase.[8]

A similar number of penicillinase-producing strains of *H. influenzæ* was found in the present study, but we also isolated another 7 strains which showed an apparent inherent diminished sensitivity to ampicillin on repeated testing with a carefully controlled inoculum. Penicillinase activity was not found in any of these strains despite concentration of the cells and their disruption by sonication.

The existence of two distinct mechanisms of resistance to ampicillin in isolates of *H. influenzæ* poses problems in sensitivity testing. Detection of strains which produce β-lactamase is simple, and the failure of ampicillin in the treatment of infections caused by these strains is well documented.[2,18] However, inherent resistance is less easily shown because of the unsuitability of disc methods for testing antibiotic sensitivity of hæmophilus[9,19] and of the need to control carefully the inoculum size in dilution techniques of MIC determinations in this species.[20] Also, it is not possible at present to predict response to therapy of infection caused by strains which vary in the extent of their inherent resistance to ampicillin. The concentration of ampicillin required to inhibit these strains is distributed over a range of values, starting from that which inhibits sensitive strains to a level as high as 8 mg/l. The degree of inherent resistance in *H. influenzæ* which would be necessary to jeopardise the response of patients with respiratory infections to treatment with ampicillin remains to be determined by further clinical and laboratory observations.

We thank Dr Douglas Smith and Dr Peter Robertson for their helpful criticisms of the paper.

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PATHOGENESIS AND NATURAL COURSE OF PRIMARY OSTEOPOROSIS

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Summary A new concept of the pathogenesis and natural course of osteoporosis is described, based on histomorphometric evaluation of iliac crest bone biopsies of 108 patients with untreated primary osteoporosis. It is suggested that primary osteoporosis spontaneously runs a three-phase course: during the initial period of negative bone balance, bone mass falls below the normal range; during the second phase bone mass remains consistently below age-related normal values; in the third phase restitution of osteoporosis is introduced by a positive bone balance. It is suggested that only about a quarter of patients with a skeletal mass below the age-related normal range have true primary osteoporosis; the remaining three-quarters may have secondary forms of osteoporosis where the underlying disease responsible for the bone loss had been missed before the osteoporosis was diagnosed.

Introduction

SEVERAL diseases of organs other than the skeleton can cause secondary loss of bone, but the cause of primary osteoporosis is still unknown. Despite the variety of possible ætiologies, the pathogenesis of all forms of osteoporosis is the same—namely, a bone balance more negative than is usual for the patient's age. As long ago as 1941, Albright et al.[1] wrote: "A diminution in the bony mass results from either an increase of bone resorption or a decrease of bone formation". It seems helpful to recognise the various degrees of these changes[2] (the five different combinations of bone forma-

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